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Influence of Biliary Drainage on the Repair of Hepatic Lesions in Biliary Fibrosis

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Background. **Bilioduodenal (BD) and biliojejunal (BJ) derivation induce enterobiliary reflux and bile stasis. Decompression of the excluded loop of the Roux-en-Y (BJD) was proposed to minimize these effects. The aim of this study was to compare the influence of these three modalities of biliary bypass on hepatic lesion repair in rats with secondary biliary fibrosis.**

Materials and Methods. **Rats with 15 d of biliary obstruction underwent BD, BJ, and BJD drainage and were compared with a group submitted to simulated operation (SO) and biliary obstruction (CBO). The serum values of total and fractional bilirubin, alkaline phosphatase (ALP), and aminotransferases (AST and ALT), as well as hepatobiliointestinal excretion determined with ^{99m}Tc-Disida, were used for comparison. In addition, we used morphometric analyses to estimate the mass of the hepatocytes, bile ducts, and liver fibrosis. We also counted hepatic stellate cells (SC).**

Results. **For each of the three modalities of biliary drainage, there were significant reductions in bilirubin, AST, ALP, and the number of SCs. The recovery of the estimated mass of all histologic components occurred only after BJ and BJD; in the BD group, the estimated hepatocyte mass was reduced compared with the SO group. The residual hepatic radioactivity of ^{99m}Tc-Disida was greater in the BJD group than in the SO group.**

Conclusions. **The interposition of the jejunal loop between the biliary tree and the intestine may slow hepatobiliary clearance of radioactivity, even though it provides the resolution of cholestasis and is effective in recovering from hepatic lesions.**

Key Words: **obstructive jaundice; liver fibrosis; choledochostomy; choledochoduodenostomy; choledochojejunostomy; liver regeneration; radionuclide imaging; cholangitis; portal hypertension; hepatic stellate cells.**

INTRODUCTION

Clinical [1–3] and experimental [4–7] records show that effective biliary anastomosis reverses hepatic changes resulting from chronic biliary obstruction. In contrast, bilioduodenal (BD) and biliojejunal (BJ) anastomosis induce enterobiliary reflux and stasis in the excluded jejunal loop [7–10].

Rats with secondary biliary fibrosis that received BD showed improvement of cholestasis and anatomopathologic liver alterations; however residual hepatic fibrosis and portal hypertension remained [11]. Other studies conducted under similar hepatic lesion conditions reported recovery of hepatic excretion, histologic liver changes and portal pressure levels after BJ [5].

Comparative studies of BD and BJ that evaluated the liver and the bile ducts reported some enterobiliary reflux and contamination of the biliary duct in both modalities of derivation but with greater intensity after BD [8]. In scintigraphic analyses of hepatobiliointestinal flow, BJ was associated with residual hepatic stasis [7, 10] but showed better recovery of the hepatic lesions [8].

In contrast, the motility changes of the excluded loop of the Roux-en-Y promoted biliary stasis, bacterial

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growth, cholangitis, and hepatic fibrosis [8, 10, 12]. The biliojejunal Roux-en-Y derivation with excluded jejunal loop decompression *via* latero-lateral anastomosis with the duodenum (BJD) is an alternative treatment that may minimize the adverse effects of stasis in BJ and ensure the benefits of BD [10, 13].

Thus, the current belief that the results of biliary obstruction treatment depend more on the underlying disease and the patency of the anastomosis than upon the modality of biliary bypass is not absolute. The frequent indications of BD due to technical ease, especially regarding videolaparoscopic access, should be cautiously taken into account when considering the treatment of biliary obstruction [14–16].

In this context, the changes in the liver and bile ducts resulting from biliodigestive anastomoses should be the subject of experimental study. The objective of this study was to evaluate the effects of BD, BJ, and BJD on anatomopathologic liver lesions and on the hepatobilio-intestinal flow in rats with secondary biliary fibrosis.

MATERIALS AND METHODS

The study was approved by the Ethics and Animal Experimentation Committee of the Faculty of Medicine of Ribeirão Preto at the University of São Paulo (FMRP-USP). We used 137 adult male albino Wistar rats with initial weights ranging from 222 to 346 g. The surviving animals were divided into two groups as described below.

Distribution of the Animals into Groups and Subgroups (Fig. 1)

- Simulated operation group (SO): $n = 8$ animals. The animals in this group underwent a simulated operation involving traction of the duodenum and careful handling of the bile duct to avoid trauma. After 2 mo, we measured hepatobiliary excretion by scintigraphy with ^{99m}Tc-Disida. We also performed splenectomy, blood sampling for biochemical tests, and total hepatectomy for histologic analyses of the left lower lobe fragment.
- Biliary obstruction group (BO): $n = 33$ animals. The BO animals had two weeks of biliary obstruction and were divided into four subgroups: CBO (8 animals), BDpre (9 animals), BJpre (8 animals), and BJDpre (8 animals).

The CBO subgroup underwent the same evaluation as the SO group. After 2 wk of biliary obstruction, the animals in subgroups BDpre, BJpre, and BJDpre underwent blood sampling for biochemical evaluation and removal of a fragment of the left lower lobe of the liver for anatomopathologic study. Following these procedures, the animals received bilioduodenal, biliojejunal Roux-en-Y anastomosis, or biliojejunal Roux-en-Y anastomosis with decompression of the excluded jejunal loop by anastomosis with the duodenum. These groups were then labeled as BD, BJ, and BJD, respectively. After 2 mo of biliary drainage, these groups underwent the same evaluation as the SO group.

Surgical Technique

After 12 h of fasting, each animal was weighed, cleaned, anesthetized, positioned in the dorsal decubitus position, and submitted to trichotomy of the anterior abdominal wall. Antisepsis was performed using polyvinyl pyrrolidone iodine (dermoiodine; J. P. Indústria Farmacêutica SA Ribeirão Preto). The abdominal cavity was accessed by median laparotomy in the upper abdomen, with lateral retraction of the edges of the incision with delicate retractors. The peritoneal cavity was kept moist with saline solution to prevent drying of the viscera.

Anesthesia

For the anesthetic, we used a combination of ketamine (50 mg/kg) and xylazine (10 mg/kg) with intramuscular administration [17]. We used intraperitoneally administered thiopental (20 mg/kg) to study hepatobilio-intestinal flow [18].

Biliary Obstruction

After bile duct isolation, a ligature was placed about 5 mm from the confluence of the lobar ducts with 5-0 prolene thread (prolene blue monofilament suture; Ethicon, Inc., São José dos Campos (São Paulo), Brazil). This first ligature was followed by a second, which was placed 3 mm above the biliopancreatic junction and the transection of the bile duct between the ligatures [19].

Biliodigestive Anastomosis (Fig. 2)

All anastomoses were made using single-layer continuous sutures with Vicryl 6-0 (polyglycolic acid) thread.

- BD group: Anastomosis between the dilated bile duct opened transversally and the duodenum opened longitudinally, for an extent of 1 cm, in a latero-lateral manner.
- BJ Group: Latero-lateral anastomosis with the jejunal loop sectioned about 5 cm from the duodenojejunal angle, followed by a terminolateral jejuno-jejunal anastomosis 15 cm from the hepatojejunal anastomosis.
- BJD Group: Latero-lateral biliodigestive anastomosis with the jejunal loop sectioned about 5 cm from the duodenojejunal angle. Latero-lateral anastomosis was then performed between the excluded loop of the Roux-en-Y and the first portion of the duodenum 5 cm away from the biliodigestive anastomosis. Jejuno-jejunal anastomosis was performed 15 cm from the hepatojejunal anastomosis.

The abdominal wall was closed in the same way in all animals. The peritoneum and aponeurosis were sutured *en bloc* with a 4.0 nylon monofilament continuous suture; the skin was closed in the same manner.

Biochemical Analysis

We measured the serum levels of total bilirubin, direct bilirubin, indirect bilirubin [20], alkaline phosphatase [21], alanine aminotransferase, and aspartate aminotransferase [22].

Histologic Analyses of the Liver

The liver fragments were fixed in 10% buffered formalin for 24 h, followed by progressive dehydration. For morphometric analyses, 4 μm slices were stained with Masson's trichrome stain. For the identification of stellate cells, serial slices of 3 μm thickness underwent immunohistochemical reactions with the monoclonal anti-desmin lyophilized rat antibody (IgG1, NCL-DES-DER11; Novocastra Laboratories Ltd., Newcastle, UK) according to the manufacturer's specifications.

For the morphometric analyses, we captured images using an Axiophot microscope (Carl Zeiss, Hallbergmoos, Germany) coupled to a digital camera (Sony Minato, Tokyo, Japan). The images were transferred to a computer *via* card capture by Frame Grabber (Carl Zeiss) in the standard RGB, with 648 $\times$ 474 lines for analysis using